

CLAIMS

We claim:

1. A method for using high affinity TCRs to identify ligands comprising:
labeling high affinity TCRs;
contacting said labeled TCRs with ligands;
identifying the ligand with which the labeled TCR is bound.
2. The method of claim 1, wherein said label is selected from the group consisting of:
fluorescent compounds, chemiluminescent compounds, radioisotopes and chromophores.
3. The method of claim 1, wherein said ligands are peptide/MHC ligands.
4. A method of using high affinity TCRs to bind to a selected peptide/MHC ligand comprising:
labeling said high affinity TCRs with a label that binds to the selected peptide/MHC ligand;
contacting said labeled high affinity TCRs with cells containing MHC molecules.
5. The method of claim 4, wherein said label is selected from the group consisting of:
fluorescent compounds, chemiluminescent compounds, radioisotopes and chromophores.
6. A method for using high affinity TCRs as diagnostic probes for specific peptide/MHC molecules on surfaces of cells comprising:
labeling high affinity TCRs with a label that binds to specific peptide/MHC ligands;
contacting said TCRs with cells;
detecting said label.
7. A method for using high affinity TCRs that bind to pMHCs for diagnostic tests comprising:

labeling the high affinity TCR with a detectable label;
contacting said high affinity TCR with cells;
detecting the label.

8. The method of claim 7, wherein the number of labels present is detected.
9. The method of claim 7, wherein the location of the labels is detected in an organism.
10. The method of claim 7, wherein said label binds to specific peptide/MHC ligands, whereby cells that express specific peptide/MHC ligands are targeted.
11. The method for blocking autoimmune destruction of cells comprising:
contacting TCRs with high affinity for the site recognized by the T lymphocytes on the surface of a target cell with cells, whereby the autoimmune destruction of cells is blocked.
12. The method for using high affinity TCRs to treat disease comprising:
coupling a TCR having a high affinity for a neoplastic cell surface marker with a therapeutic compound; and
contacting said TCR with cells.
13. A method of using high affinity TCRs to inactivate pathogens comprising:
binding a molecule which is toxic to the pathogen to the high affinity TCR; and
contacting said TCR with cells that express said pathogen.
14. The method of claim 13, wherein said pathogen is selected from the group consisting of:
virus, bacteria and protozoa.
15. Soluble T cell receptors (TCRs) having higher affinity for a ligand than wild type TCRs.
16. The soluble high affinity TCRs of claim 15, wherein said ligand is a peptide/MHC ligand.

17. The soluble high affinity TCRs of claim 15, wherein said high affinity TCR is made by the method comprising: mutagenizing a TCR to create mutant TCR coding sequences; transforming DNA comprising the mutant TCR coding sequences for mutant TCRs into yeast cells; inducing expression of the mutant TCR coding sequences such that the mutant TCRs are displayed on the surface of yeast cells; contacting the yeast cells with a fluorescent label which binds to the peptide/MHC ligand to produce selected yeast cells; and isolating the yeast cells showing the highest fluorescence.
18. The soluble high affinity TCRs of claim 15 isolated by yeast display.
19. A DNA library comprising nucleic acids encoding soluble high affinity TCRs, wherein said TCRs are made by the method of mutagenizing a TCR to create mutant TCR coding sequences; transforming DNA comprising the mutant TCR coding sequences for mutant TCRs into yeast cells; inducing expression of the mutant TCR coding sequences such that the mutant TCRs are displayed on the surface of yeast cells; contacting the yeast cells with a fluorescent label which binds to the peptide/MHC ligand to produce selected yeast cells; and isolating the yeast cells showing the highest fluorescence.
20. A library of T cell receptor proteins displayed on the surface of yeast cells which have higher affinity for the peptide/MHC ligand than the wild type T cell receptor protein, wherein said library is formed by mutagenizing a T cell receptor protein coding sequence to generate a variegated population of mutants of the T cell receptor protein coding sequence; transforming the T cell receptor mutant coding sequence into yeast cells; inducing expression of the T cell receptor mutant coding sequence on the surface of yeast cells; and selecting those cells expressing T cell receptor mutants that have higher affinity for the peptide/MHC ligand than the wild type T cell receptor protein.
21. A method for cloning the gene for a high affinity TCR mutant into a system that allows expression of the mutant on the surface of T cells comprising:

- mutating TCRs to create high affinity TCR mutants;
cloning said TCR mutants into a vector;
transfecting the vector into T cells;
expressing the high affinity TCR mutant on the surface of T cells.
22. The method of claim 20, further comprising:
selecting those T cells that are activated by a peptide/MHC ligand more than the wild type.
23. The method of claim 20, wherein the transfected/infected T cells are used for recognition of selected peptide-bearing MHC cells.
24. T cells made by the method of claim 20.
25. A method for using high affinity T Cell Receptors (TCRs) to detect ligands comprising the steps of:
labeling high affinity TCRs;
contacting said labeled high affinity TCRs with ligands;
detecting the presence of the label thereby detecting the ligand to which the labeled high affinity TCR is bound wherein the high affinity TCR exhibits a dissociation constant for the ligand greater than about 10^7 .
26. The method of claim 25 where the high affinity TCR exhibits a dissociation constant for the ligand from about 10^7 to about 10^{10} .
27. The method of claim 25 wherein the ligand is a peptide/MHC ligand.
28. The method of claim 27 wherein the peptide/MHC ligand is on the surface of a cell.
29. The method of claim 25 wherein the peptide is a superantigen.

30. The method of claim 25 wherein the label is selected from the group consisting of:
fluorescent compounds, chemiluminescent compounds, radioisotopes and chromophores.
31. The method of claim 25 wherein the high affinity TCR carries one or more mutations in a CDR.
32. The method of claim 31 wherein the one or more mutations are in CDR3 α or CDR3 β .
33. A method for using high affinity T Cell Receptors (TCRs) to detect ligands comprising the steps of:
labeling high affinity TCRs;
contacting said labeled TCRs with ligands;
detecting the presence of the label thereby detecting the ligand to which the labeled TCR is bound wherein the high affinity TCR carries one or more mutations in a CDR.
34. The method of claim 33 wherein the one or more mutations are in CDR3 α or CDR3 β .
35. The method of claim 33 wherein the ligand is a peptide/MHC ligand.
36. The method of claim 33 wherein the peptide/MHC ligand is on the surface of a cell.
37. The method of claim 33 wherein the label is selected from the group consisting of:
fluorescent compounds, chemiluminescent compounds, radioisotopes and chromophores.
38. A method for blocking autoimmune destruction of target cells comprising the step of
contacting target cells with a soluble mutant TCR with high affinity for the site
recognized by a T lymphocyte on the surface of the target cells, whereby the autoimmune
destruction of the target cells is blocked, wherein the soluble, high affinity mutant TCR
exhibits a dissociation constant for the ligand greater than about 10^7 .

39. The method of claim 38 wherein the high affinity mutant TCR exhibits a dissociation constant for the ligand between about 10^7 and 10^{10} .
40. The method of claim 38 wherein the high affinity TCR is a mutant carrying one or more mutations in a CDR.
41. The method of claim 40 wherein the high affinity TCR is a mutant carrying one or more mutations in CDR3 α or CDR3 β .
42. A method for blocking autoimmune destruction of target cells comprising the step of contacting target cells with a soluble mutant TCR with high affinity for the site recognized by a T lymphocyte on the surface of the target cells, whereby the autoimmune destruction of the target cells is blocked, wherein the soluble, high affinity mutant TCR carries one or more mutations in a CDR.
43. The method of claim 42 wherein the high affinity TCR is a mutant carrying one or more mutations in CDR3 α or CDR3 β .
44. A method for using high affinity TCRs to kill an undesirable cell comprising the steps of: coupling a TCR having a high affinity for cell surface marker of the cell with a therapeutic compound to form a therapeutic TCR derivative; and contacting the therapeutic TCR derivative with the undesirable cell, wherein the high affinity TCR exhibits a dissociation constant for the cell surface marker of the undesirable cell greater than about 10^7 .
45. The method of claim 44 wherein the high affinity TCR exhibits a dissociation constant for the ligand between about 10^7 and 10^{10} .

46. The method of claim 44 wherein the high affinity TCR is a mutant carrying one or more mutations in a CDR.
47. The method of claim 42 wherein the high affinity TCR is a mutant carrying one or mutations in CDR3 α or CDR3 β .
48. A method for using high affinity TCRs to kill an undesirable cell comprising the steps of: coupling a TCR having a high affinity for cell surface marker of the cell with a therapeutic compound to form a therapeutic TCR derivative; and contacting the therapeutic TCR derivative with the undesirable cell, wherein the high affinity TCR carries one or more mutations in a CDR.
49. The method of claim 48 wherein the high affinity TCR carries one or more mutations in CDR3 α or CDR3 β .
50. A method of binding a high affinity TCR to a cell carrying a selected peptide/MHC ligand on the cell surface which comprising the steps of: providing a mutant TCR exhibiting a dissociation constant of greater than about 10^7 for the selected peptide/MHC ligand; labeling the high affinity TCR; contacting the labeled high affinity TCRs with a sample containing cells carrying one or more peptide/MHC ligands on the cell surface to bind the high affinity TCRs to selected peptide/MHC ligands present in the sample.
51. The method of claim 50 wherein the mutant TCR exhibits a dissociation constant between about 10^7 to 10^{10} for the selected peptide/MHC ligand.
52. The method of claim 50 wherein the mutant TCR carries one or more mutations in CDR.
53. The method of claim 51 wherein the mutant TCR carries one or more mutations in

CDR3 α or CDR3 β .

54. A method of binding a high affinity TCR to a cell carrying a selected peptide/MHC ligand on the cell surface which comprising the steps of:
providing a mutant TCR having high affinity for the selected peptide/MHC complex and carrying one or more mutations in a CDR;
labeling the high affinity TCR;
contacting the labeled high affinity TCRs with a sample containing cells carrying one or more peptide/MHC ligands on the cell surface to bind the high affinity TCRs to selected peptide/MHC ligands present in the sample.
55. The method of claim 54 wherein the mutant TCR carries one or more mutations in CDR3 α or CDR3 β .
56. A method for cloning the gene for a high affinity TCR mutant into a system that allows expression of the mutant on the surface of T cells comprising the steps of:
mutating TCRs to create high affinity TCR mutants which exhibit a dissociation constant for their cognate ligand of at least about 10^7 ;
cloning said TCR mutants into a vector;
transfecting the vector into T cells; and
expressing the high affinity TCR mutant on the surface of T cells.
57. The method of claim 56, wherein the transfected T cells are used for recognition of selected peptide-bearing MHC cells.
58. The method of claim 56 wherein the high affinity TCR mutants carry one or more mutations in a CDR.
59. The method of claim 58 wherein the high affinity TCR mutants carry one or more mutations in CDR3 α or CDR3 β .

60. A method for cloning the gene for a high affinity TCR mutant into a system that allows expression of the mutant on the surface of T cells comprising the steps of:
mutating TCRs to create high affinity TCR mutants carrying one or more mutations in a CDR;
cloning said TCR mutants into a vector;
transfecting the vector into T cells; and
expressing the high affinity TCR mutant on the surface of T cells.
61. The method of claim 60, wherein the transfected T cells are used for recognition of selected peptide-bearing MHC cells.
62. The method of claim 60 wherein the high affinity TCR mutants carry one or more mutations in CDR3 α or CDR3 β .
63. T cells made by the methods of claim 56.
64. A DNA sequence encoding a mutant high affinity TCR exhibiting a dissociation constant of greater than about 10^7 for its cognate ligand.
65. The DNA sequence of claim 64 wherein the TCR mutant exhibits a dissociation constant between about 10^7 and 10^{10} for its cognate ligand.
66. The DNA sequence of claim 64 wherein the TCR mutant carries one or more mutations in a CDR.
67. The DNA sequence of claim 66 wherein the TCR mutant carries one or more mutations in CDR3 α or CDR3 β .

68. A DNA sequence encoding a mutant high affinity TCR carrying one or more mutations in CDR.
69. The DNA sequence of claim 68 carrying one or mutations in CDR3 α or CDR3 β .
70. A therapeutic TCR derivative which comprises a soluble high affinity single chain TCR coupled to a therapeutic compound.
71. The therapeutic TCR derivative of claim 70 wherein the therapeutic compound is an anticancer agent, a therapeutic radionuclide or a cytotoxic protein.
72. The therapeutic TCR derivative of claim 70 wherein the TCR specifically binds to a pathogen infected cell.
73. The therapeutic TCR derivative of claim 72 wherein the therapeutic compound is a molecule that is toxic to a pathogen.
74. The therapeutic TCR derivative of claim 70 wherein the high affinity TCR exhibits a dissociation constant greater than about 10^7 for its cognate ligand.
75. The therapeutic TCR derivative of claim 70 wherein the high affinity TCR carries one or more mutations in a CDR.
76. A method for treating disease in a patient comprising the steps of:
removing wild-type T cells from the patient;
transforming the T cells with the vector that expresses a high affinity TCR mutant, to express the high affinity TCR in the T cells;
returning the transformed T cells to the patient;
wherein the transformed T cells are activated to a greater extent than the wild type T cells of the patient.

77. The method of claim 76 wherein the high affinity TCR mutant exhibits a dissociation constant greater than 10^7 for a selected ligand.
78. The method of claim 76 wherein the high affinity TCR mutant carries one or more mutations in a CDR.
79. T cells made by the methods of claim 60.
80. A pharmaceutical composition comprising a high affinity TCR in a pharmaceutical carrier wherein the high affinity TCR exhibits a dissociation constant for a ligand of greater than about 10^7 .
81. A method of using the composition of claim 80 comprising administering the composition to a patient.
82. The method of claim 6, wherein said detecting step is performed by flow cytometry.
83. The method of claim 7, wherein said detecting step is performed by flow cytometry